Timolol Effects on Aqueous Humor Dynamics in Eyes of Anesthetized Rats

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ABSTRACT

Anterior chambers of the eyes of male rats were cannulated under pentobarbital anesthesia for intracameral infusions of balanced salt solution (BSS) and intraocular pressure (IOP) recording. Blood pressure was recorded from a femoral artery. IOP was recorded during a 2-h intracameral infusion composed of a constant component (0.05 μl/min) and a periodic component (0.25 μl/min), cycling at 4 min on and then 4 min off. After a 20-min baseline period, 1 drop of timolol (0.5%) or BSS was applied to the cornea and repeated 1 h later. Intracameral infusions of BSS and 0.05% timolol were also compared. Topical timolol slightly delayed the BSS-induced IOP rise (p < .05). Complex demodulation and the estimated gain parameter of a second-order transfer function fit to the periodic responses revealed that topical timolol also reduced (p < .05) passive outflow resistance. Intracameral timolol markedly delayed the BSS-induced rise in IOP. Initially, timolol decreased both outflow impedance and nonresistive components (p < .05) of IOP, but these effects dissipated by 2 h when IOPs were similar. In all experiments, within-group blood pressure was unchanged. Topical and intracameral timolol have different effects on IOP. The data support the opinion that, in vivo, timolol acts at β-receptors that control both outflow impedance and nonresistive mechanisms, probably vascular, to lower IOP.

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ABBRVIATIONS: BSS, balanced salt solution; IOP, intraocular pressure; BP, mean arterial blood pressure; A o, passive resistance to aqueous humor synthesis; corneal epithelial and endothelial cells, pigmented epithelium of the retina, trabecular meshwork, and ciliary muscle and epithelium (Moroi and Lichter, 1996). Although β-receptors at multiple ocular sites might contribute to therapy, differences in pharmacokinetic characteristics at each site make it difficult to evaluate the simultaneous contribution of all intraocular β-receptors to pharmacodynamic action. Reduction of aqueous humor synthesis by β-receptor blockade at the ciliary epithelium is frequently cited as the mechanism for reducing IOP (Bartels et al., 1980; Bartels, 1988; Hoffman and Lefkowitz, 1996). Effects of β-receptor blocking drugs on ocular hemodynamics may also be of importance (Findl et al., 1997; Schmetterer et al., 1997; Yatsuksa et al., 1998). In particular, color Doppler analysis techniques have demonstrated that vasculature at the optic nerve head is responsive to topical timolol (Baxter et al., 1992).

Techniques to obtain a broad overview of the pharmacodynamic effects of β-receptor blockade in the eye are limited. Ideal methods to assess simultaneously the multiple components of ocular dynamics and effects of drugs in living eyes are lacking. At steady state, IOP represents an equilibrium among three constantly interacting processes: rate of aqueous humor formation, passive outflow resistance along the trabecular and uveoscleral outflow pathways; RP, residual pressure, unexplained by analysis, that encompasses aqueous humor synthesis rate and episcleral venous pressure.
trabecular meshwork and uveoscleral pathway, and a vascular effect, termed episcleral venous pressure (Davson, 1980). Also, the volume of blood perfusing the eye and its intracocular distribution are closely related to blood pressure (BP) and IOP (Yatsuka et al., 1998).

Methods to determine pressure/volume characteristics of the eye may be broadly categorized as static or dynamic (Sears, 1960; Reuben et al., 1985; Balaban et al., 1997). Static methods require steady-state conditions, thereby precluding detection of rapid and simultaneous adjustments in synthesis, resistance to outflow, and vascular effects. Dynamic methods were employed by Eisenlohr and Langham (1962) and Viennstein and Cowan (1969) to assess the elastic properties of the eye as a function of pressure. These early studies showed that sinusoidal infusion (constant rate with a sinusoidal oscillation) schemes were feasible for eye research. To our knowledge, this approach, which does not require steady-state conditions, has not been used to differentiate acute drug effects on resistive versus nonresistive aspects of aqueous humor dynamics.

Recently, we employed a dynamic approach in anesthetized rats using a procedure permitting continuous IOP recording during infusion of the anterior chamber with balanced salt solution (BSS) at a constant volume, and a superimposed cyclic infusion (Balaban et al., 1997). Low-rate volume infusions of BSS raise IOP after a brief lag (Sears, 1960; Gaasterland et al., 1978; Reuben et al., 1985), and drug effects on this increase can be assessed by topical application to the cornea or as part of the infusate. The algorithm we used allows changes in IOP to be evaluated in terms of resistive (trabecular and uveoscleral drainage) versus nonresistive components (Balaban et al., 1997). The present report describes effects of timolol (Timoptic, Merck & Co., West Point, PA) as a representative $\beta$-receptor blocking drug used in glaucoma management. Two routes of drug delivery were tested: topical application to the cornea and continuous intracameral infusion.

**Materials and Methods**

**General.** Adult male Sprague-Dawley rats (300–400 g) were kept in a temperature and humidity controlled room with a 12 h light/dark cycle. They had food and water ad libitum, and were cared for in accordance with the Guide for the Care and Use of Laboratory Animals, Department of Health, Education, and Welfare/National Institutes of Health publication number 186 to 23 (1985). The experimental protocol was approved by the Institutional Animal Care and Use Committee of the College of Medicine, Pennsylvania State University.

**Experiment Initiation.** Rats were anesthetized with sodium pentobarbital (45 mg/kg i.p.). Body temperature was maintained by incandescent lamps. Catheters were placed in a femoral artery and vein, and flushed as needed with heparinized (50 IU/ml) saline. The former was used for continuous BP measurement, whereas the latter was used to maintain anesthesia. When needed, pentobarbital was infused slowly until BP stabilized near 80 mm Hg. The procedure to cannulate the anterior chamber has been described in detail elsewhere (Palm et al., 1995; Searles et al., 1996). Briefly, the tip of a 27-gauge needle was inserted and fixed in the anterior chamber of one eye, and a tubing/manifold system allowed IOP recording and the connection of two infusion pumps. The BSS for intracameral infusion (Merlis, 1940) has been used for multiple biologic applications, including central nervous system infusions (Severs and Daniels-Severs, 1973), ocular infusions (Palm et al., 1995), and piggyve suspensions for transplantation (Weiss et al., 1978). It was routinely filtered with a 0.22-μm Millipore filter (Millipore Corp., Milford, MA) just before use.

**Protocol.** A 20-min baseline was allowed to ensure that BP and IOP were stable. Then two infusion pumps were activated for 2 h. The first pump infused BSS continuously at a rate of 0.05 μl/min. The second pump delivered BSS in a cyclic manner at a rate of 0.25 μl/min. A timer automatically activated this pump for 4 min, then turned it off for 4 min. Thus, the “average” volume delivered to the anterior chamber was 0.175 μl/min, which approximates 25% of the aqueous synthesis rate for rats (Searles et al., 1996). Prior work determined that these rates provide a sufficient magnitude of oscillation for estimation of outflow impedance (Balaban et al., 1997). Two sets of experiments were performed. The first experiment used rats infused as described into the anterior chamber, and 1 drop of BSS or timolol (Timoptic, 0.5%) was applied to the surface of the cornea and repeated 1 h later. The drops were applied from Timoptic droptainers to a distant portion of the cannula shaft, such that the drop ran down the needle and wet the entire surface of the eye. A photograph of a cannulated rat eye appears in Searles et al. (1996) to aid in visualizing the drop instillation. The second experiment used BSS or timolol (Timoptic), diluted with BSS to 0.05%, as anterior chamber infusates. The 10-fold dilution was based on data that showed that after topical application of timolol to albino rabbit eyes, corneal drug concentration was approximately 10-fold higher than aqueous humor concentration, and the apparent $T_{1/2}$ of the drug in the cornea and aqueous humor was similar (Mishima, 1982).

**Data Acquisition and Analysis.** Ultralow volume transducers for BP and IOP were connected to a Keithley model 570 data acquisition system (Keithley Data Acquisition Div., Tauton, MA) and a computer that sampled the transducer outputs every second, stored data on a hard drive for off-line analysis, and displayed pressures on a CRT screen for real-time monitoring (for details, see Morrow et al., 1992; Searles et al., 1996). IOP and BP data were partitioned into 10-min time bins and evaluated by two-way analysis of variance (ANOVA) with time as a repeated measure. Significant F ratios ($p < .05$) were assessed by Tukey’s test. To derive estimates of resistive and nonresistive changes, the IOP data stored on the computer were synchronized to the time when the pumps were turned on. Data from the 8-min cycles were then assessed by a standard complex demodulation approach to determine the magnitude and phase angle of the IOP responses at the fundamental frequency of 0.125 cycles/min (Bloomfield, 1976). Next, the transfer function of cycle-by-cycle responses to the periodic infusion was calculated.

Two assumptions are implicit in the process. First, it is assumed that the IOP control system is linear to a first approximation, such that the IOP responses to the mean infusion rate and the IOP responses to the periodic component are linearly superimposed. Second, it is assumed that the coefficients of the transfer function are constant within a given cycle of stimulation. The validity of these assumptions, the data filtering routine, and the specific equations used have been discussed in detail previously (Balaban et al., 1997).

The gain parameter of the transfer function, $A_o$, reflects passive outflow resistance, i.e., the combined resistance to aqueous humor drainage by trabecular and uveoscleral pathways (units: mm Hg $\mu$l $^{-1}$ min $^{-1}$). The residual pressure (RP) of IOP that is unexplained by the transfer function for the periodic component reflects nonresistive changes in synthesis rate and/or episcleral venous pressure. Changes in $A_o$ and RP were evaluated over thirteen 8-min cycles by ANOVA and least significant difference tests (Systat, Evanston, IL).

**Results**

**Topical Application of Timolol.** Figure 1 presents the IOP and BP data that compare the two topical applications of timolol and BSS during the constant + cyclic intracameral infusions. For the IOP data, the $F$ ratios for treatment and interaction were not significant ($F < 1, p > .05$), whereas the repeated measure for time was highly
significant ($F > 25, p < .001$). It should be noted that timolol is known not to lower IOP in the unconscious states like sleep in humans (McCannel et al., 1992) or anesthetized animals (Bartels et al., 1980). Baseline IOPs, at the time of the first BSS or timolol application, were similar: $10.2 \pm 0.6$ versus $10.0 \pm 1.2$ mm Hg, respectively. Withingroup comparisons showed that the infusion-induced rise in IOP was significant ($p < .05$) 30 min after topical BSS, rose further to a maximum at 70 min ($p < .05$), and remained unchanged ($p > .05$) until the infusion stopped. The infusion-induced rise in IOP, after the first drop of timolol, first became significant ($p < .05$) at 60 min, increased gradually ($p < .05$) to a maximum value at 100 min, and then remained unchanged ($p > .05$) until the end of the infusion. ANOVA of the BP data revealed no significant $F$ ratios for treatment, time, or interaction ($p > .1$).

Figure 2 contains the data for passive resistance ($A_o$) and nonresistive (RP) changes during these experiments. Repeated-measure ANOVA of the $A_o$ estimates, over the $13 \times 8$-min cycles of infusion, revealed significant ($p < .05$) $F$ ratios for treatment, cycle, and interaction. The major differences are best illustrated by comparing cycles 1 to 7 versus cycles 7 to 13. Cycles 1 to 7 revealed no significant treatment effect ($p > .05$), a strong cycle effect ($p < .001$), and an interaction effect ($p < .05$). Resistance rose in both groups, and the interaction reflected a decrease in $A_o$ by timolol at cycle 7. During the latter part of the experiment (cycles 7–13), the ANOVA revealed a strong treatment ($p < .001$), a small cycle ($p = 0.04$), and no interaction ($p > .1$) effects. A small, further rise in $A_o$ continued ($p < .05$); but $A_o$ suppression by timolol was maintained ($p < .05$).

Repeated measures ANOVA for RP, also shown in Fig. 2, yielded nonsignificant $F$ ratios ($p > .01$) for treatment and interaction effects, but a highly significant effect of cycle ($F > 23, p < .001$). That is, a small, gradual increase in nonresistive dynamic components of IOP occurred in both groups, reaching a plateau by the eighth cycle.

**Intracameral Infusion of Timolol.** IOP and BP data from this experiment are shown in Fig. 3. ANOVA for the IOP data yielded significant ($p < .05$) $F$ ratios for treatment, time, and interaction effects. The IOP of the timolol rats was less than control until 120 min. Baseline IOP of timolol rats was slightly but significantly ($p < .05$) less than control, because some drug likely diffused into the eye after the cannula tip was inserted and the 20-min baseline IOP recording started. Within the control group, IOP rose significantly at 20 min ($p < .05$), reached an apparent peak at 50 min ($p < .05$), and no further increase ($p > .05$) occurred during the infusion. Then IOP partially ($p < .05$) recovered toward baseline and tended ($p > .05$) to rise slightly. Within the timolol group, IOP was first elevated 60 min after the infusion ($p < .05$). IOP rose slightly from 60 to 100 min ($p < .05$). This was an apparent peak, because the within-group ANOVA indicated that IOP was unchanged from 80 min until the pumps were turned off ($p > .05$). IOP of timolol-treated rats returned more completely ($p < .05$) to their initial baseline, without a tendency for a secondary rise as noted in control rats. ANOVA of the BP data did not yield significant $F$ ratios for treatment, time, or interaction ($Fs < 2.2, p > .05$).

Figure 4 presents data for passive resistance ($A_o$) and nonresistive (RP) components of ocular dynamics. ANOVA for $A_o$ yielded $F$ ratios for treatment, cycle, and interaction, respectively, of 0.4 ($p > .2$), 4.1 ($p < .001$), and 2.2 ($p < .05$). The following information emerged from analysis of the interaction. $A_o$ rose in both groups during the first four cycles, and the timolol values were less than BSS ($p < .05$). $A_o$
remained unchanged (p > .05) in BSS rats from cycles 4 to 13. An abrupt increase occurred in the Ao of timolol-treated rats between cycles 4 and 5. Passive outflow resistance was similar in both groups from cycles 5 to 10 (p > .05). Ao then rose in timolol-treated rats and significantly (p < .05) exceeded values of BSS-treated rats from cycles 11 to 13.

ANOVA of the RPs (the nonresistive changes in ocular dynamics) generated significant (p < .001) F ratios for treatment (F = 22), cycle (F = 4.1), and interaction (F = 2.2). The BSS rats had a gradual rise in RP until cycle 6 (p < .05) and then reached a plateau. RP in timolol-treated rats was unchanged (p > .05) during the first seven cycles and was less (p < .05) than control values. Then, RP in the timolol group began to rise and was similar (p > .05) to the control values from cycles 11 to 13.

**Discussion**

IOP represents a dynamic equilibrium among three interacting factors; synthesis rate, resistance to outflow, and a vascular effect often termed episcleral venous pressure (Davson, 1980). The cardiovascular system must provide an adequate blood volume that must be properly distributed within the eye to support these processes (Harris et al., 1994; Findl et al., 1997; Schmetterer et al., 1997). Distortions in IOP control and ocular perfusion may contribute to the pathophysiologic mechanism(s) of glaucoma (Moroi and Lichter, 1996).

Therapy of glaucoma is usually directed at the components of aqueous humor dynamics that determine IOP. β-receptor blockade lowers elevated IOP, and the mechanism is generally considered to involve a reduction in the rate of synthesis of aqueous humor (Bartels et al., 1980; Bartels, 1988; Hoffman and Lefkowitz, 1996). However, because the measured variable, IOP, reflects an equilibrium among multiple components, it is difficult to establish directly the relative participation of each individual determinant of IOP.

A well-recognized phenomenon that arises when pressure/volume relationships in the eye are distorted by BSS infusions, even at low volumes, is that BSSs raise IOP by an occult mechanism, known as the “washout effect”, perhaps involving alterations in the extracellular matrix (Sears, 1960; Gaasterland et al., 1978; Reuben et al., 1985). This effect occurred during the periodic infusion protocol described herein. The rise in IOP of the BSS groups in the two experiments was accompanied by increases in both resistive and nonresistive mechanisms (Figs. 2 and 4). Prior experiments indicated that the nonresistive mechanism does not include acetazolamide-sensitive aqueous humor synthesis. Both Ao and RP appeared to reach a peak, and the rise in Ao appeared to have a more rapid onset. Both topical and directly infused timolol modified aspects of intraocular dynamics changed by these low-volume sinusoidal infusions. It should be noted that the stable mean BPs within groups suggests that mean perfusion pressure of the eye was unaffected, but this is not a direct measure of volume or distribution of ocular blood. Removal of the vasodilatory action of β-receptors in the eye by timolol might selectively alter per-
fusion of individual structures within the eye. Schmetterer et al. (1997) reported that topical application of different β-blocking drugs did not uniformly affect intraocular hemodynamics in humans.

Timolol, applied topically, reduced by about 50% the BSS-induced rise in passive resistance to outflow. No alterations in IOP or nonresistive mechanisms were observed. This may explain the drug-induced delay of the expected infusion-induced rise in IOP. The first statistically significant (p < .05) rise in IOP within the BSS and timolol-treated rats differed. These times were 20 min for BSS rats versus 60 min for timolol-treated animals. The lack of statistically significant (p > .05) IOP and RP changes when Aβ was unstable (interaction term from repeat-measure ANOVA was p < .05) illustrates the utility of complex demodulation and transfer function analyses that do not require steady state. All of the forces determining IOP were probably not at equilibrium. Topical timolol could delay, but not prevent, the rise in IOP associated with the “washout” effect.

When infused directly into the anterior chamber, timolol reduced IOP as well as passive resistance and nonresistive mechanisms, which may include aqueous synthesis and episcleral venous pressure. Most of the rise in IOP occurred between 20 to 50 min in the BSS group and between 60 to 100 min in the timolol group. IOPs of both groups were similar by the end of the infusion. The timolol-induced decrease in Aβ ended by cycle 5, whereas the decrease in nonresistive mechanisms ended at cycle 11. Thus, like topical timolol, intracameral infusion of the drug delays but does not prevent the “washout effect”, even though the drug infusion was continuous.

It should be noted that these experiments comparing topical and intracameral timolol were acute, and should not be interpreted beyond the time of data collection. Single doses were used by each route, so dose-response for changes in ocular impedance and nonresistive mechanisms are not yet available. Thus, the relative importance of the importance of timolol on resistive and nonresistive components of ocular dynamics are not completely resolved. However, because an efficacious dose was used for each route and the method assesses ocular dynamics without the need for steady-state or pressure/volume clamping, we believe the data accurately reflect the initial events in ocular dynamics triggered by timolol in the living eye.

In summary, the present report documents that periodic infusions of low volumes of BSS into the rat eye evoked an IOP rise by a “washout effect”. This method of raising IOP provides a potentially important method for evaluation of drugs that may lower the high IOP associated with glaucoma. Complex demodulation and analysis of a second-order transfer function permitted insight into the mechanism of action of a widely used antiglaucoma drug, timolol, in the rat eye in vivo. Applied topically, the drug delayed the IOP increase and lowered resistance to outflow along trabecular-uveoscleral pathways. When infused intracameraly, timolol produced a longer lasting delay in the IOP rise by decreasing resistive and nonresistive intraocular dynamics, indicating multiple sites of timolol action within the eye. The drug-induced delay in resistive mechanisms ended before the delay in nonresistive components, thereby documenting different temporal properties. This experimental model appears useful for determining the acute mechanisms of antiglaucoma drugs during infusion-induced increases in IOP and permits separate analysis of resistive and nonresistive changes in aqueous humor dynamics.

References

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